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On the Possibilities of a Scanning Electron Microscopic Examination of Ephemeroptera, Odonata and Trichoptera larvae

By

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The purpose and antecedents of the investigations

As the aquatic insects impopulate our waters generally in great numbers of species and individuals, and are rather large-sized, their use as indicator organisms in the practice of water quality tests seems by all means expedient. However, the fulfillment of this evident task of theirs is still hindered by several objective difficulties at present.

The majority of the information on aquatic insects most important for us is, namely, connected primarily with the biology of their aquatic larvae. In many instances, however, the determination of the larvae is extremely uncertain, often not even possible. In general, we do not even know the biology of the larvae as our requirements would necessitate it; or, even if there are data pertaining hereto at disposal in the literature, on account of the differences in the ecological conditions, the adoption of these references is often not feasible. For solving these problems at least in part, opportunities for applying several methods are presented. One may simultaneously collect adults and larvae, and raise the latter. Still, besides these expedient yet most time-consuming methods we also have to search incessantly after the new and modern procedures which afford hopes of making the determination of the larvae of aquatic insects easier, and which also further a more thorough knowledge of the ecology of such larvae.

In 1977/78 the author had opportunity to work with the Scanning Electron Microscope type SEOL 50A of the Biological Department Group of the Eötvös Loránd University and to make an attempt at the taxonomic and ecological application of the SEM technique.

On the method

The principle and main field of application of the electron microscope operating with the scanning electron ray are treated of in a great number of handbooks (1, 2), etc. Of the principle of the method the author might only mention that

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this technique constitutes, in numerous respects a transition between the various methods of light microscopy and the traditional electron microscope. Its great advantage is that by its means stereoscopic pictures can be made, and the highly demanding work of sectioning applied in transmission electron microscopic examination is spared.

The disadvantages of the Scanning Electron Microscope, namely that its resolving power is less than the one of the traditional electron microscope, and that it serves in the first place for examining the surface structures, do not give rise to problems in the taxonomic and ecological investigations, indeed, it is often expressly advantageous.

The significance of SEM is being discovered in a number of fields of biology again and again. One can agree with V. A. HEYWOOD's (1971) statement that the SEM technique will soon become a routine procedure, and that in an even greater measure than transmission electron microscopy (2).

First results and the possibilities of further application

Since it was an experiment unique in Hungarian hydrozoology up to now in question here, the author had to lay the main emphasis upon solving the problems of methodology. Examinations had to be conducted for deciding which of the methods of preparation could be adopted with the animal groups in question.

Even the simplest method proved sufficient with the examined species at all times. With all three taxonomic groups we could adopt the preparation as follows: 1) conveying alcoholic material – through a series of dilutions – into distilled water; 2) ultrasonic shaking (for 5 – 7 minutes, depending on the quality of the material); 3) drying under cover for 2 – 3 hours; 4) mounting on to a copper stud and steaming with gold.

After the methodological problems have been solved, our initial results can be surveyed by animal groups.

In the course of the examination of the mayfly larvae it proved true that the certainty of the determination of the larvae could be increased by the SEM technique only if in the single genera a material of a significant number of species and individuals was at disposal, which had been unambiguously determined by the traditional microscope.

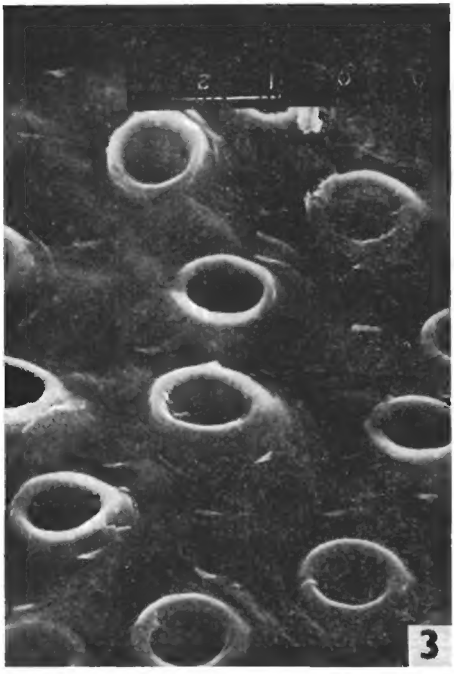
A problem of methodology was presented in the province of enlargements above 1000 – 1500 ×, where morphological structures already difficult to observe by the light microscope and even less known as yet appeared to view.

With the larvae of *Cloeon dipterum* living among the vegetation, the chitin shield showed a tiling-like pattern.

In the mud-inhabiting *Caenis robusta* and *Caenis horaria* larvae this chitin structure of the surface was of spinous arrangement (Photograph 1).

In taxonomic respect the SEM examination of the formations of the chitin structure and of the sense organs on the surface did not afford much that could be used; it is only the number and location of the individual types of hairs – in general, mechanical sense organs – that can be employed later on as taxonomic characters (Photograph 2).

On the gills and on the chitin cuticle below same of *Caenis robusta* and *Caenis horaria* „craterlike" sense organs are to be found, which belong with the chemical sense organs (Photographs 3 and 4).



Photographs 1—4. *1:* 4th abdominal segment of *Caenis horaria*, dorsal side (2450 x); *2:* 2nd leg of *Cloeon dipterum*, last segment, ciliary hairs (1480 x); *3:* crater-like sense organs under the gills of *Caenis robusta* (2450 x); *4:* crater-like sense organ under the gill of *Caenis robusta* (8200 x).

Structures similar to these have been discovered also in the larvae of the American mayfly species *Caenis diminuta* as well as in other insect larvae, and an osmoregulative function – connected with chloride ion secretion – is attributed to them (5, 6).

Making use of the experience acquired previously in the examination of the mayfly larvae, we conducted exploratory examinations in the larvae of some Odonata and Trichoptera species.

In the larvae of the dragonfly the chitin framework is smooth, nearly free of any structure, only here and there is its monotony broken by vast spine- and hair formations. In taxonomic respect, the observation of the fine details may bring new results in this field.

In the Trichoptera larvae – both by species and by stages of development – the chitin structure of the surface showed extreme diversity; therefore further comparative material needs to be collected of them.

By way of a summary: it can be stated relying upon our examinations that the SEM technique can be profitably used in the taxonomic and ecological examinations of each of the three animal groups. It can be used for obtaining information on the fine details of the taxonomic characters and also in ecological respect numerous further application possibilities present themselves.

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